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GLUCOREGULATION AFTER CANINE ISLET TRANSPLANTATION: CONTRIBUTION OF INSULIN SECRETORY CAPACITY, INSULIN ACTION, AND THE ENTERO-INSULAR AXIS

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☐ **Abstract — The physiological glucoregulatory mechanisms after islet transplantation have been incompletely investigated. We studied the insulin secretory capacity (ISC) by intravenous arginine stimulation during 35-mM glucose clamps, insulin action during hyperinsulinemic euglycemic clamps, and mixed-meal stimulation at 6–9 mo after intrasplenic islet autotransplantation in 8 dogs, as compared with 30 controls. The enteroinsular axis in the recipients was examined by infusion of porcine glucose-dependent insulinotropic polypeptide (GIP) and human glucagon-like peptide-1 (GLP-1) (7–36 amide) under 8.5-mM glycemic clamp conditions in order to mimic the postprandial glycemia after transplantation. The grafts comprised 25% of the native islet mass, and the ISC likewise averaged 25% of the control value. The postprandial insulin response, in contrast, had increased to 140% after transplantation—albeit with a concomitant glucose excursion to approximately 8.5 mM. Insulin action declined on average by 45% posttransplant. The ISC correlated both with the postprandial glucose excursion and insulin action in the grafted dogs. Insulin action did not correlate with the postprandial glucose excursion. Infusion of GIP had no effect, but GLP-1 nearly doubled glucose-stimulated insulin. Thus, a hyperglycemia-enhanced insulinotropic effect of GLP-1, and perhaps other gut hormones, may account for the difference in the insulin response to the intravenous and oral challenges. Because the ISC reflects the engrafted islet mass and appears to be the primary determinant of glucose tolerance, transplantation of higher islet doses should allow prolonged near-normal glucoregulation—at least, in the autotransplant setting. © 1997 Elsevier Science Inc.**

☐ **Keywords — Islet of Langerhans; Transplantation; Glucose-dependent insulinotropic polypeptide; Glucagon-like peptide-1.**

INTRODUCTION

Islet transplantation is a promising approach to reinstate normal glucose control in insulin-dependent diabetic patients. However, to what extent the physiological glucoregulatory mechanisms are preserved after islet transplantation has been incompletely investigated. Detailed functional data following successful clinical islet transplantation are scant (4,8,14), and the insulin secretory capacity and physiological glucoregulation have received limited attention in experimental studies, as well (17,20,21,24,25,28). We, therefore, investigated the insulin secretory capacity by intravenous arginine stimulation during 35-mM glucose clamps, the action of insulin during hyperinsulinemic euglycemic clamps, and the efficacy of physiological meal stimulation, in 8 long-term islet autotransplanted dogs. We recently presented data suggesting that hyperglycemic potentiation of the enteroinsular axis may be an important mechanism to limit postprandial glucose excursions after islet transplantation (24). We further demonstrated that freshly isolated islets still do respond in vitro to physiological stimulation with the insulinotropic gut hormones (incretins) GIP and GLP-1 (23). As yet, however, it remains to be elucidated whether these incretin effects are preserved after long-term transplantation of islets. In addition, therefore, we examined the insulinotropic effects of low-dose infusion of GIP and GLP-1 in the transplanted animals under hyperglycemic clamp conditions that mimic the postprandial approximately 8.5 mM glycemia, observed in our islet recipients.

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MATERIALS AND METHODS

Animals

Experiments were performed, in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and institutional guidelines, in 38 adult, female, outbred beagles (Harlan, Zeist, The Netherlands) weighing 8–18 kg. Eight dogs underwent total pancreatectomy and intrasplenic islet autotransplantation, the other dogs served as controls. The animals were maintained on a regular diet of semiliquid dog food (50 energy% carbohydrate, 20 energy% fat, and 30 energy% protein; Complete Dog Food D-B, Hope Farms, Woerden, The Netherlands) twice daily and had free access to water. After islet transplantation the diet was supplemented with 2 g/day protease-lipase-amylase pellets (Organon, Oss, The Netherlands).

Islet Transplantation

After a 24-h fast, total pancreatectomy was performed for islet isolation and autotransplantation in dogs, anesthetized as described previously (24). Islets were isolated from the pancreas by intraductal perfusion with Hanks' balanced salt solution (Flow Laboratories, Irvine, UK) containing 1600 U/mL collagenase type V or XI (Sigma, St. Louis, MO), digestion for 8–20 min at 37°C, and gentle trituration of the tissue at 4°C. The tissue was resuspended in the bottom layer of step density gradients (1.095, 1.085, 1.075, and 1.045 g/mL) of 70 kDa dextran (Sigma) in Hanks' solution, and purified islets were collected from the two uppermost layers after centrifugation at 500 × *g* for 20 min. The islets were autotransplanted in the spleen of the animal by reflux via two splenic vein tributaries, while clamping the splenic pedicle and short gastric vessels. The total volume of transplanted islets and the purity (relative islet volume) of the graft were assessed by morphometry (22). The islet dose per kg body weight averaged 3679 ± 849 islet equivalents (IEQs), which corresponds to approximately 25% of the average native islet mass of 14668 IEQs/kg in 14 control pancreases as assessed by stereology (24). The purity of the grafts was 70 ± 10%.

Glucoregulation Studies

The tests were performed in the controls and 6–9 mo posttransplant in the islet recipients, after an overnight 18-h fast with an interval of at least 2 days. Animals appeared to be in good condition, and had normal stools and steady body weights—recipients: 11.0–12.6 kg (92 ± 4% of preoperative weight)—throughout the studies. Serial weekly assessment of the fasting and 2-h postprandial plasma glucose levels in the recipients during 2 mo preceeding the metabolic studies demonstrated fasting normoglycemia—individual glucose values <6.4 mM (upper limit of 95% confidence interval in controls)—and a stable mean 8.4–9.4 mM postprandial glycemia in the transplant group each week. The antecedent overall mean 2-h postprandial glycemia was 8.8 ± 1.2 mM.

The blood sampling and infusion procedures have been described (24). Plasma insulin was radioimmunoassayed using dog insulin as standard (24) and glucose was assayed with a Glucose Analyzer 2 (Beckman, Palo Alto, CA).

The intravenous glucose tolerance test (IVGTT), insulin secretory capacity test, and meal test have been detailed previously (24). Briefly, IVGTT was performed to determine both glucose clearance (k_G) and the acute insulin response above baseline from 0–3 min after bolus infusion of 0.5 g glucose per kg body weight. For assessment of the insulin secretory capacity plasma glucose was raised and maintained at approximately 35 mM for 80 min by variable rate infusion of a 40% glucose solution, guided by on-line plasma glucose analysis every 5 min to manually control the glucose infusion rate. Blood for insulin assay was drawn as shown in Fig. 1. The secondary insulin response to steady-state 35 mM glycemia was expressed in the mean increment from 45 to 50 min above baseline. At 50 min, a 2 g arginine hydrochloride bolus was administered. The insulin secretory capacity was expressed in the mean acute insulin increment from 52 to 55 min over the mean 45–50 min prestimulus insulin level. A test meal consisting of 500 mL of the regular semiliquid meal was ingested by the animals within 15 min. Blood was drawn as shown in Fig. 1 for assessment of the integrated responses above baseline of plasma glucose and insulin during 2 postprandial hours.

Insulin action was measured by a two-step hyperinsulinemic euglycemic clamp. After taking baseline samples for glucose and insulin assay at −15, −10, −5, and 0 min, insulin (Actrapid; Novo, Copenhagen, Denmark) was infused at 10 mU/min from 0 min, and at 50 mU/min from 90–180 min, during which a variable infusion of a 20% glucose solution kept plasma glucose at the mean fasting level, guided by glucose analysis as described above. Samples for plasma insulin assay were drawn every 10 min from 60 to 90, and from 150 to 180 min, for calculation of the index of insulin action ($10^2 \cdot L \cdot kg^{-1} \cdot min^{-1}$ per pM) as described by Finegood and coworkers (2). Insulin clearance was expressed in the high-dose insulin infusion rate per kg body weight, divided by the corresponding mean insulin level, during the final 30 min.

The insulinotropic effects of GIP (n = 7) and GLP-1 (n = 4) were studied in the transplanted animals during approximately 8.5-mM glycemic clamps in order to

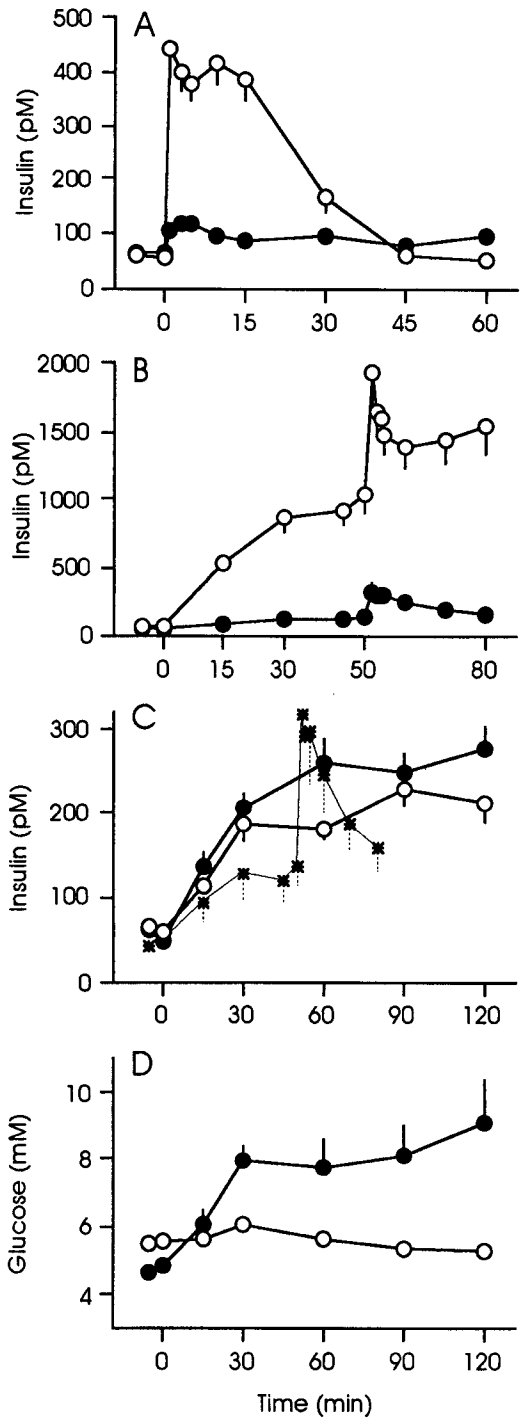

Fig. 1. Response curves in 30 normal control dogs (open circles) and eight dogs with intrasplenic islet autografts at 6–9 mo after transplantation (closed circles) of plasma insulin following (A) an intravenous glucose bolus, (B) an intravenous arginine bolus during an intravenous 35 mM glucose clamp, and of plasma (C) insulin and (D) glucose after a mixed meal. The posttransplant insulinemia following arginine stimulation during the 35 mM glucose clamp is plotted again as an overlay (asterisks) in panel C for comparison with the postprandial insulinemia.

mimic the postprandial glycemic conditions. The recipients not studied were either excluded because fasting hyperglycemia had developed ($n = 1$), or were no longer available because our studies were performed in two consecutive series of experiments, and the GLP-1 study was introduced first in the second series. Each animal underwent three glycemic clamps on separate days, one without and the others with the simultaneous coinfusion of GIP or GLP-1. After taking baseline samples a variable 40% glucose infusion was started at 20 mL/h to raise and maintain plasma glucose at approximately 8.5 mM for 110 min, guided by glucose analysis as described above. From 50 to 80 min either the vehicle was coinfused in the contralateral hindleg, or 1.75 pmol · kg^{-1} · min^{-1} of synthetic porcine GIP or synthetic human GLP-1 (7–36 amide) (Peninsula, Merseyside, St. Helens, UK) dissolved in 0.9% NaCl containing 0.1% bovine serum albumin (fraction V, 35%-solution, Sigma). Net peptide content rather than gross weight was used for calculation of the dose. Blood was drawn for plasma insulin assay as shown in Fig. 3. The insulinotropic effects of the peptides were quantitated by comparison of the mean insulin level and glucose infusion rate from 65–80 min.

Statistical Analysis

Integrated responses were calculated using the trapezoidal rule, and weighted by dividing by the respective periods. Logarithmic transformation of data was used when appropriate to normalize the distribution of the data. Results are expressed as mean ± SEM, and differences between means were analyzed by Student's *t*-test for paired or unpaired data and considered not significant at $p > 0.05$.

RESULTS

For each animal, the fasting glucose and insulin values were calculated as means of the baseline levels of all the tests. The mean fasting glycemia (5.3 ± 0.1 vs. 5.3 ± 0.1 mM) and insulinemia (62 ± 3 vs. 57 ± 2 pM) were similar in the control and experimental animals, respectively. Glucose clearance (k_G) during IVGTT (2.4 ± 0.1 vs. 0.9 ± 0.1 %/min) amounted posttransplant to 40% of the normal value ($p < 0.0001$). Figure 1 shows the insulin response curves during IVGTT (A), the intravenous 35 mM glucose clamp with arginine stimulation (B), the meal test (C), and the glucose response to a meal (D).

Both the acute insulin response from 0–3 min at IVGTT (305 ± 37 vs. 37 ± 8 pM; Fig. 1A), and the secondary insulin response from 45–50 min during the 35 mM glucose clamp (922 ± 120 vs. 81 ± 20 pM; Fig.

Table 1. Two-step sequential hyperinsulinemic euglycemic clamp

Parameter	Controls	Grafts
Insulin action index ($10^2 \cdot L \cdot kg^{-1} \cdot min^{-1}$ per pM)	38 ± 5	21 ± 3*
Insulin clearance ($mL \cdot kg^{-1} \cdot min^{-1}$)	42 ± 4	34 ± 3
First insulin level (pM)	157 ± 9	172 ± 13
Second insulin level (pM)	732 ± 63	840 ± 74

Values are means ± SEM.
*$p < 0.05$ vs. controls.

1B) amounted to approximately 10% of the control values ($p < 0.0001$). The insulin secretory capacity—the acute insulin response from 52–55 min to arginine bolus stimulation during the 35 mM glucose clamp (Fig. 1B)—averaged posttransplant 25% of the control value (693 ± 77 vs. 172 ± 44 pM; $p < 0.0001$). In contrast, the postprandial insulin response (Fig. 1C) had increased posttransplant to approximately 140% of the normal response (119 ± 10 vs. 164 ± 15 pM; $p < 0.05$). The posttransplant insulinemia after a meal corresponded to the posttransplant peak insulinemia during the insulin secretory capacity test, which is shown again for direct comparison as an overlay graph in Fig. 1C. Postprandial glycemia (Fig. 1D) reached a mean 8.5 mM after transplantation and the glucose increments amounted to 0.2 ± 0.1 vs. 2.9 ± 0.7 mM ($p < 0.0001$) in the control and experimental group, respectively. As shown in Table 1, insulin action in the grafted dogs averaged 55% of the normal value ($p < 0.05$). Insulin clearance was not affected by islet transplantation.

The insulin secretory capacity of the individual grafts correlated significantly with both the postprandial glucose excursion ($p < 0.001$; Fig. 2A), and the index of insulin action ($p < 0.05$; Fig. 2B) in the graft recipients. The index of insulin action did not significantly correlate with the postprandial glucose excursion (data not shown).

Infusion of GIP in the islet recipients did not effect the insulinemia nor the glucose uptake—the glucose infusion rate required to maintain the 8.5 mM glycemia (data not shown). The insulinotropic effect of GLP-1 infusion during 8.5 mM glucose clamps in the islet recipients is illustrated in Fig. 3.

As shown in Table 2 insulinemia during the final 15 min of GLP-1 infusion increased to a mean 175% of the corresponding insulinemia during the glucose clamp without coinfusion of the peptide. Glucose uptake paralleled the increment in insulin levels.

DISCUSSION

We studied glycemic control in long-term fasting-normoglycemic dogs with intrasplenic autografts of purified islets. The poor beta-cell response and glucose intolerance following an intravenous glucose challenge in the graft recipients corroborated previous studies of dog autografts (17,21,24) and streptozotocin-diabetic rats—even after isografting the average normal islet mass (25). Because antecedent chronic hyperglycemia has been shown to impair the insulin response to intravenous glucose (15), probably both the repetitive postprandial hyperglycemia and the 75% reduction of the beta-cell mass after transplantation contributed to the 90% reduction of intravenous glucose-stimulated insulin in our graft recipients. The insulin secretory capacity—as

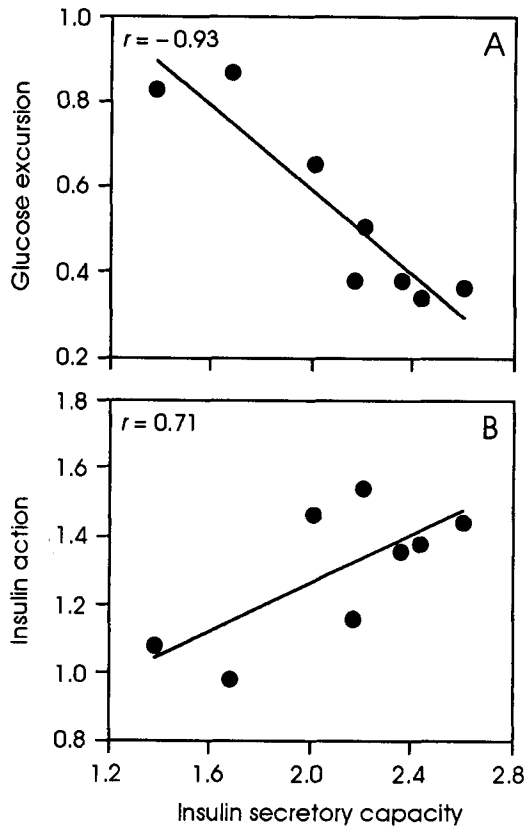

Fig. 2. Correlation in the graft recipients of the insulin secretory capacity as determined by intravenous arginine stimulation at 35 mM glycemia, vs. (A) the postprandial glucose excursion and (B) insulin action, after logarithmic transformation of the data.

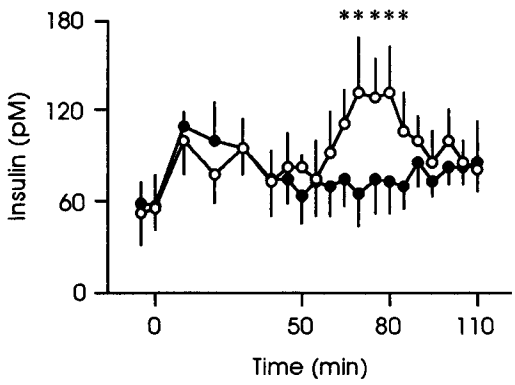

Fig. 3. Plasma insulin response during intravenous 8.5 mM glucose clamps with (open symbols) or without (closed symbols) coinfusion of GLP-1 (1.75 pmol · kg^{-1} · min^{-1}) from 50–80 min, at 6–9 mo after islet autotransplantation. Asterisks indicate significant ($p \leq 0.05$) differences between insulin levels at each time point.

assessed by arginine stimulation under marked hyperglycemic conditions—is, in contrast, probably not affected by antecedent hyperglycemia (7,15), and has been shown to be a sensitive indicator of the reduction in beta-cell mass in partial pancreatectomized and diabetic subjects (26,27). The posttransplant approximately similar 75% reduction of both the islet mass and the insulin secretory capacity, indicated the latter parameter to be a sensitive gauge of the beta-cell mass in our model as well. With the assumption of (near-) maximal beta-cell stimulation during the insulin secretory capacity test (10,26) the posttransplant similar insulinemia after a meal indicated that the graft was (near-) maximal stimulated postprandially as well. This conclusion is substantiated by the (negative) correlation of the insulin secretory capacity vs. the postprandial glucose excursion in the transplanted animals. These findings confirm and extend our previous reports of the remarkable difference between the insulin response to an intravenous and oral challenge after islet transplantation in rats and dogs (24,25). Apart from the dose of transplanted islets, purity of the graft appeared to be also important for the functional outcome. The posttransplant insulin secretory capacity in this study of purified islet grafts nearly doubled, and postprandial glucose tolerance was superior, compared to the data we obtained in a study of partially purified grafts that comprised a similar dose of islets (24)—indicating that acinar contamination of an islet graft may impede the engraftment and survival of islets.

We hypothesized that the graft's hyperinsulinemic response to a meal, as opposed to the hypoinsulinemic response to an intravenous challenge, may be attributed to hyperglycemic potentiation of the enteroinsular axis after meals—rather than the hyperglycemia per se. In vitro, we recently demonstrated glucose-dependent potentiation of insulin secretion from freshly isolated perifused canine islets by physiological levels of human GLP-1, and—albeit approximate 10-fold less—by physiological levels of porcine GIP (23). Similar progressively greater potentiating effects of GIP and GLP-1 on glucose-stimulated insulin release at increasing ambient glucose levels have been reported in vivo (6,11)—however, as yet, not after islet transplantation. We therefore investigated the insulinotropic effects of low-dose infusion of porcine GIP and human GLP-1 during 8.5 mM glucose clamps in order to mimic the postprandial glycemic conditions after islet transplantation. The absence of an incretin effect of GIP in the present study corroborated our previous in vitro finding of a 10-fold smaller effect of GIP compared to GLP-1, and because porcine GIP differs from canine GIP, the data should most probably be taken to indicate that porcine GIP cannot be used to study low-dose physiological effects in the dog, rather than indicating no role of GIP as an incretin in our model. Human GLP-1, in contrast, may be identical to canine GLP-1 because the structure of GLP-1 has been shown identical in all mammals studied so far (23). The insulinemia during the 8.5 mM glucose clamps in our grafted dogs nearly doubled during infusion of GLP-1 at a dose that is known to lead to near-physiological plasma GLP-1 levels in man (12), and which has been shown to be less insulinotropic at lower (6–7 mM) glucose levels in normal dogs (5). A hyperglycemia-enhanced insulinotropic effect of GLP-1—and perhaps other gut hormones, as well—may, therefore, account for the marked difference in the insulin response to the intravenous and oral challenges. The fact that the glucose infusion rate had to be increased to maintain the glucose level at 8.5 mM during GLP-1 administration, is well

Table 2. Insulinotropic effect of GLP-1 during 8.5 mM glucose clamps in the grafted dogs

Parameter	No GLP-1	GLP-1
Insulin level (pM)	72 ± 19	126 ± 17*
Glucose level (mM)	8.5 ± 0.2	8.4 ± 0.3
Glucose infusion (mg · kg^{-1} · min^{-1})	5.8 ± 2.0	9.4 ± 2.8†

Values are means ± SEM of the individual mean insulinemia, glycemia, and glucose infusion rate during the final 15 min infusion of GLP-1 (from 65–80 min).
*$p < 0.01$, †$p < 0.05$.

compatible with its glucose lowering effect in both diabetic and normal subjects (1,3,9), and probably may be attributed to its effects on islet function (1,13), rather than on insulin sensitivity as in IDDM (3).

Apart from the insulin secretory deficit, insulin resistance probably also contributed to the impaired glucose tolerance in our graft recipients. However, correlation of the graft's insulin secretory capacity with both the postprandial glucose excursion and insulin action in the grafted animals—and no significant correlation between the latter two parameters—would suggest the graft's beta-cell mass to be the primary determinant of the glucose intolerance. Studies in partially pancreatectomized animals and newly diagnosed patients with insulin-dependent diabetes mellitus, likewise, indicate that insulin resistance accompanies a reduced insulin secretory capacity, and may increase as the residual insulin secretory capacity decreases (16,27,29). In contrast, no insulin resistance, or correlation of the insulin secretory capacity and insulin action—as assessed by an exogenous insulin-modified intravenous glucose tolerance test—was observed by Tobin and co-workers in islet autografted dogs (20). The basis for this discrepancy may be manifold—both, perhaps a larger mass of engrafted islets, an interindividually less varying insulin secretory capacity, and the different assay of insulin action in the latter study, may account for the different findings. In a recent hyperinsulinemic-euglycemic clamp study of high-dose islet transplanted insulin-dependent patients (8), an only slightly impaired inhibition of hepatic glucose production was observed in a group of patients with well-functioning grafts, and clear-cut evidence for insulin resistance was obtained only in a group of patients with partial or nonfunctioning grafts. Thus, the accumulated evidence would suggest that (near-) normal insulin action can be attained provided that a sufficient islet dose is engrafted.

It should be noted that postprandial hyperglucagonemia and pancreatic polypeptide deficiency may also have contributed to the impaired glucose tolerance in our model, as reported previously (24). Further, the largely or exclusively (vagal) cholinergic-mediated preabsorptive insulin response, which is important for postprandial glucose tolerance (8,18,19), is known to be absent or drastically curtailed after islet transplantation in rats (19,25), and may be largely absent in our animals as well, because persistent cholinergic denervation of the graft is observed in our model (24).

We conclude that the islet mass deficit after transplantation results in a similar insulin secretory deficit that appears to be the major determinant of glucose tolerance and insulin action. A hyperglycemia-enhanced insulinotropic effect of GLP-1, and perhaps other gut hormones, may largely account for the marked difference in the insulin response to intravenous and oral challenges after transplantation. After transplantation of a suboptimal islet mass, postprandial hyperglycemic potentiation of the enteroinsular axis may, on the one hand, restrain the postprandial glucose excursion, and, on the other hand, lead to (near-) maximal stimulation of postprandial insulin—which, tentatively, might eventually lead to functional failure of the graft. Because the beta-cell mass appears to be the primary determinant of glucoregulation, we conclude that transplantation of higher doses of purified islets should allow prolonged near-normal glycemic control—at least, in the autotransplant setting.

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